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13. ABSTRACT (Maximum 200 Words)

Bleeding from an artery is difficult to control due to the high pressures found in the arterial system. Hemorrhage is especially problematic in penetrating wounds where the bleeding source may not be apparent. Tourniquets that are routinely used to treat such wounds can cause multiple complications. We are developing a device which, when exposed to aqueous solutions, rapidly generates pressure in a confined space. In this report, we summarize the design and testing of a prototype device. The "biohemostat" is composed of a flexible outer membrane, which surrounds a hydrophilic, super-absorbent polymer. The outer bag is made from an electrospun mat of Ethylene-vinyl acetate co-polymer. The electrospun mat is very flexible, durable (stretching to 10 times its original length), biocompatible and porous. Its relative degree of hydrophobisity is overcome by incorporating a percentage of EVOH either as a blend or composite. The hydrophilic polymer used in the prototype device is composed of polyacrylic acid derivatives or copolymers. When the device is placed in aqueous solutions it rapidly absorbs fluid, expands and develops significant pressure in a confined space. Although swelling of such polymers is dependent on the nature of the aqueous solution (i.e. Varies with pH, ionic strength, protein content, etc.) the decreases in absorption caused by these parameters have been easily overcome by increasing the amount of hydrophilic polymer. We have met all engineering goals and can develop 90 mm Hg pressure within 180 seconds in a confined space. The goal is to place this device directly into a wound and develop counter pressure to aid in hemorrhage control. By developing pressure directly on the bleeding site, it may be possible to avoid the crush injuries and ischemic damage associated with tourniquet use.

14. SUBJECT TERMS
Arterial hemorrhage, penetrating wounds, hemostat, wound dressing, tourniquet, hemostasis, acute treatment, limb sparing

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Table of Contents

D. INTRODUCTION

Ballistic injury is a primary mode of trauma in combat. Such injuries can be associated with rapid blood loss due to vascular disruption. In the Vietnam conflict, ten percent of wounds to the extremity were associated with major artery injury.[1] While bleeding from compressible vessels may respond to direct pressure, blood loss from deep muscular branches such as those from the profunda femoris artery may be severe.[2] Despite increasingly aggressive surgical treatment, limb salvage has not improved[3], and death from hemorrhagic shock remains a problem even in very healthy individuals.[4] Combat vascular injuries continue to result in a 12 to 30 percent amputation rate depending on the involved vessel.[5]

Described more than 2000 years ago as an adjuvant to surgical amputation[6], tourniquets have become a primary initial treatment of injuries with associated high pressure bleeding. Unfortunately, tourniquet utilization can be associated with a variety of complications including nerve injuries, distal ischemia, compartment syndromes, post-tourniquet syndrome, and pulmonary embolus.[7,8] A major consequence of these complications is an increased risk of limb wastage. Despite these potential complications, combat as recent as the 1991-92 Croatian conflict has verified the ability of tourniquets to delay shock in lower extremity arterial injuries.[9]

Recent developments in the field of hemostatic agents have raised the possibility of alternative treatment of vascular bleeding. The development of virally inactivated fibrin sealant and its documentation as a useful adjuvant to multiple types of surgery have been major advances.[10,11] The effectiveness of fibrin glue in speeding hemostasis along vascular graft suture lines[12], presaged its testing as an adjuvant to surgery in the treatment of complex hepatic injury.[13] Alternate formulations of fibrinogen and thrombin containing dressings[14] and dry fibrin sealant dressings[15] have prompted studies of these dressings in pig models of vascular injury[14] and grade V liver injury.[15,16] Dry fibrin sealant dressing was recently shown to be more effective than standard gauze in decreasing bleeding and maintaining blood pressure in ballistic injury.[17]

While the development of "dry" products has increased their potential as alternatives to tourniquets for battlefield treatment, several potential problems remain. First, these products are very expensive. Second, although virally inactivated, the fibrinogen they contain comes from multiple human donors and cannot be considered totally safe in terms of pathogen transmission. Third, these products must be held in place until bleeding stops or the material may simply wash out of the wound. This is especially true when the bleeding is brisk as with arterial involvement. The need for a tourniquet alternative that is effective, inexpensive, lacks viral risk, and can be easily administered by an army medic is obvious.

Superabsorbent polymers are crosslinked hydrophilic polymer networks with the ability to absorb large quantities of pure water, saline or physiological solutions.[18,19] Superabsorbent polymers can absorb large amounts of water or other fluids and swell up to thousands of times their own weight in aqueous media. The absorbed water is retained within the network even under considerable pressure.[20,21] Superabsobent polymers have been utilized in a variety of applications including drug delivery systems, absorption pads, consumer care products, disposable diapers, hygienic napkins, biomedical materials, soft contact lenses, supports for catalysts, soil components for agriculture and horticulture, gel actuators, water blocking tapes, and artificial snow for winter sports.[22-25]

The electrostatic spinning (electrospinning) process is an attractive approach for processing polymer biomaterials because it offers the opportunity for control over material morphology, porosity, and composition using simple equipment. In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets. At sufficiently high polymer concentrations, chain entanglements in melts allow production of continuous fibers. The fibers are produced by charging the liquid to 5000-30,000 Volts vs. a ground a short distance away. This leads to injection of the charged liquid from the catheter type electrode and capture of the forming polymer on a device placed between the catheter and the electrical ground.

Electrospinning is a cost effective method for producing fibrous polymer mats with fiber diameters ranging from 0.01 μm to several tens of μm.[26-29] Such materials may be useful for many applications in medicine such as wound dressings and scaffolds for tissue engineering.[30-32] The simplicity of the electrospinning process itself, the ability to control the fiber diameter and overall porosity of the resulting mat, and the ability to incorporate therapeutic compounds into the mats during spinning, afforded the prospect of preparing useful polymer systems for controlled drug delivery. While flat mats represent an attractive form for topical delivery applications, other shapes (e.g., tubes) can be can be constructed using different target geometries.

We used the electrospinning technique to manufacture the outer (highly permeable) bag of the biohemostat device. We had previously shown the utility of this approach in the delivery of drugs such as the antibiotic tetracycline Hydrochloride.[33] Due to it's well know biocompatibility, ethylene-vinyl acetate copolymer was selected for this biohemostat applications.[34-36] EVA has been used in many biomedical applications including controlled release of drugs and macromolecules such as immunoglobulin G.[37,38] In some applications, such as controlled release of insulin, EVA has been used as an implantable polymer.[39] Its use in the field of dental diseases has also been reported.[40,41]

In this report, we detail work accomplished during the first two years of a grant (DAMD 17-01-1-0691) from the United States Army. Progress toward the design, construction and testing of a new hemostatic device composed of a porous outer bag containing superabsorbent polymers is summarized. All pre-determined engineering performance criteria have been met.

E. BODY

Work Accomplished will be detailed by the specific goals listed in our approved statement of work.

The original device concept involved a relatively small, flexible bandage that would be placed directly in the wound. Once in place, liquid within the wound would rapidly penetrate the bandage and be absorbed by super-absorbent polymers contained within the bandage. As a consequence of fluid absorption, the bandage would expand and develop pressure within the wound to aid in hemorrhage control.

Development of the Outer (encasement membrane) Material(Goals 6 and 7)

Based on this simple design, the outer material of the device had to meet certain functional criteria (Goals 6 and 7). The outer coating must be: flexible, porous, biocompatible, wettable, and durable. We chose electrospinning (Figure 1 and 2) as the process by which we would prepare various sheets of candidate polymers for initial testing. Electrospinning is simple, inexpensive and

allows the production of polymer sheets of varying size, thickness and shape. Ethylene Vinyl Acetate (EVA) was initially chosen as a candidate material due to its high flexibility (figure 3), large pore size (see figure 4) and biocompatibility. As can be seen by scanning electron microscopy (figure 5), electrospun EVA is composed of a continuous string of polymer (i.e. there are no obvious polymer endpoints). This property increases structural integrity and therefore decreases the possibility of device rupture during use or removal.

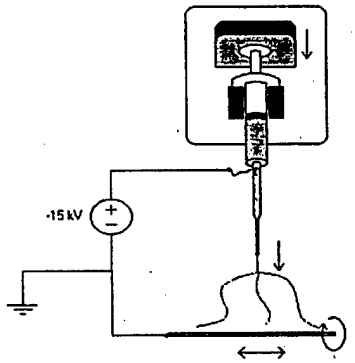


Figure 1. Schematic of Electrospinning

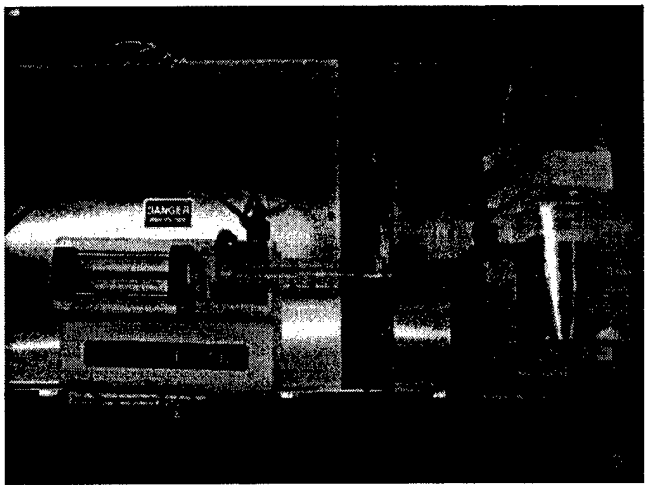


Figure 2.

Figure 3.

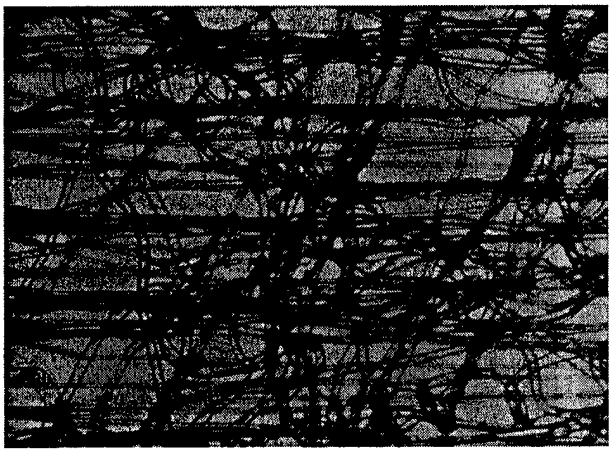
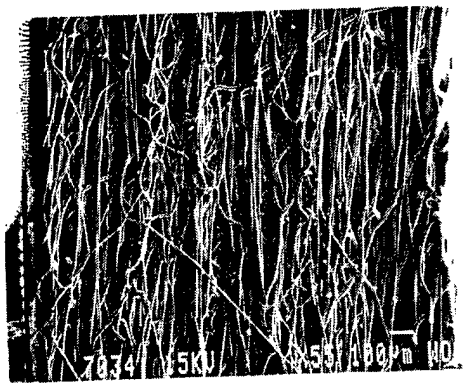


Figure 4.

Unfortunately, EVA is not extremely wettable. This property slowed initial swelling of prototype devices (Figure 6). Once swelling began, it proceeded rapidly due to the opening of large pores in the EVA network. The period prior to rapid swelling was too excessive to allow EVA to function as the outer device material.

Addition of hydroxyl (OH) groups to EVA produces Ethylene Vinyl Alcohol (EVOH), which is dramatically more wettable but much less flexible (Figure 7). By combining alternating

layers of EVA and EVOH a material was produced with the requisite outer-shell properties (see figure 8).



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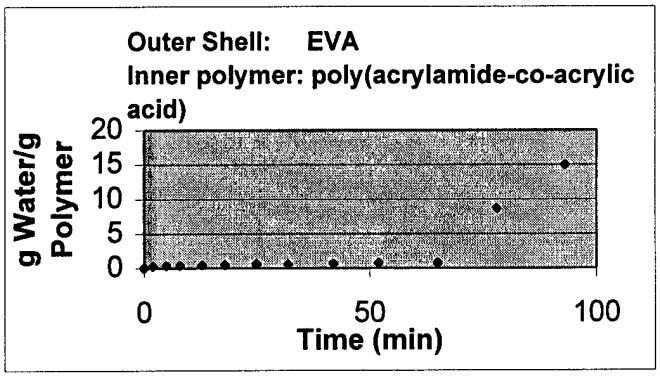

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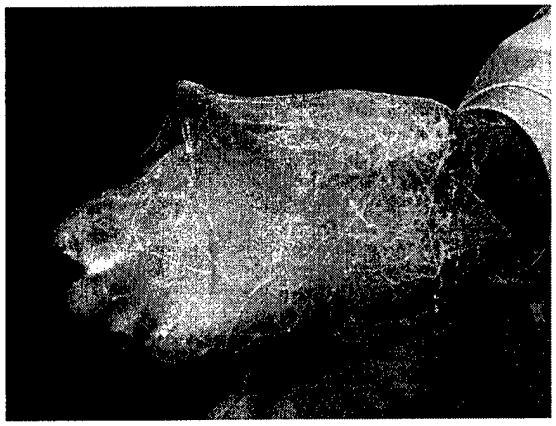

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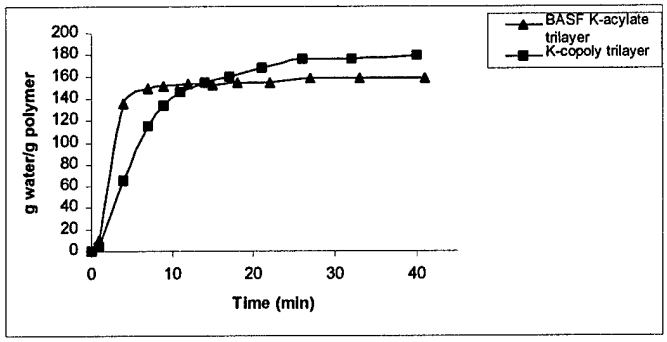


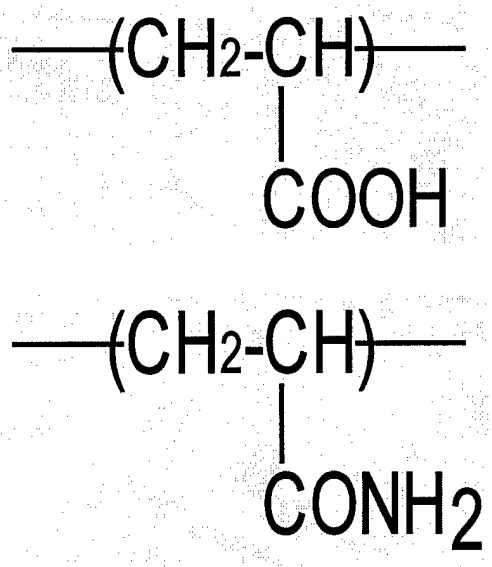
Figure 8.

Selection and optimization of Super-Absorbent Inner Polymer Materials (Goals 1 and 2)

Testing of several candidate commercially available super-absorbent polymers identified poly(acrylic-co-acrylamide) (Figure 9) as an appropriate hydrophilic polymer for the BioHemostat device. The speed of swelling was optimized by the addition of potassium salt (Figure 10, compare to figure 6) and by polymer neutralization (figure 11). Direct comparison of the neutralized polymer and the potassium salt (Figure 12) lead to the acceptance of the Poly(acrylic-co-acrylamide) potassium salt as the best candidate for further device development (figure 13). This polymer rapidly expands, absorbing up to 1,000 times its weight in water.

The absorption characteristics of all super-absorbent polymers are to some degree dependent on the nature of the aqueous environment. That is to say, absorption is altered by increasing ionic strength, changes in pH and the presence of proteins. Since the BioHemostat will be utilized at an

ionic strength of 0.15M, a pH of 7.4 and in the presence of large amounts of protein, the ability of prototype devices to absorb salt solutions, plasma and blood were therefore studied. As anticipated, increased ionic strength and the presence of protein slowed and decreased absorption. This was most easily overcome by simply increasing the amount of absorbent polymer contained within the devices (Figure 14). Subsequent testing in whole blood (figures 15 and 16) confirmed the ability of the device to absorb in this environment.



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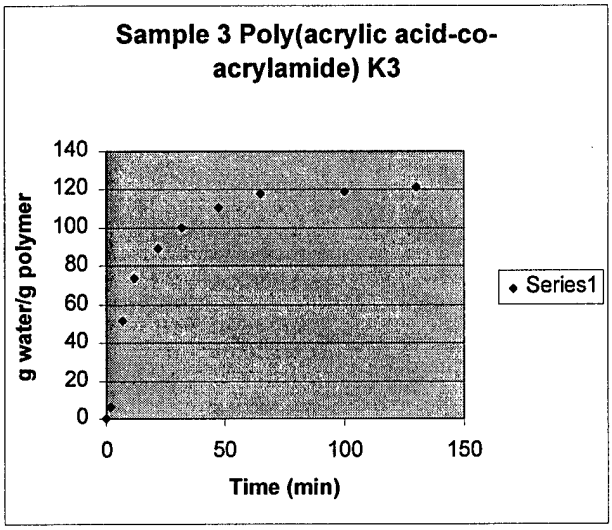

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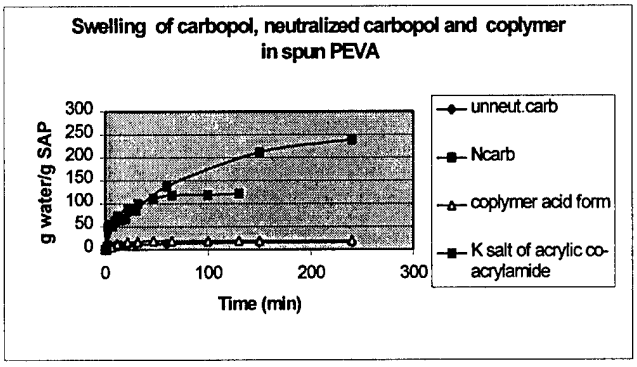

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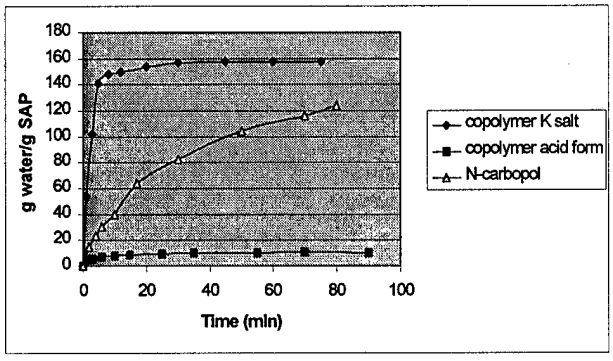

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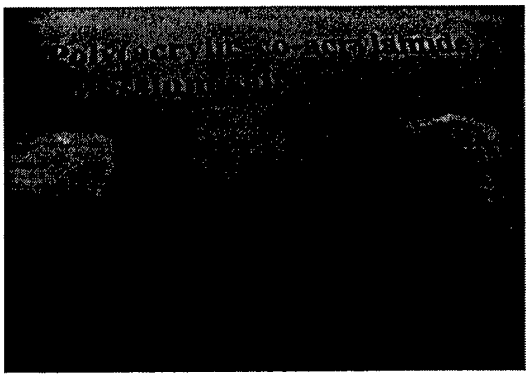

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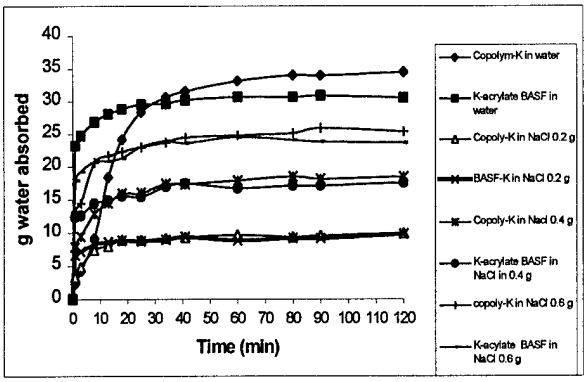

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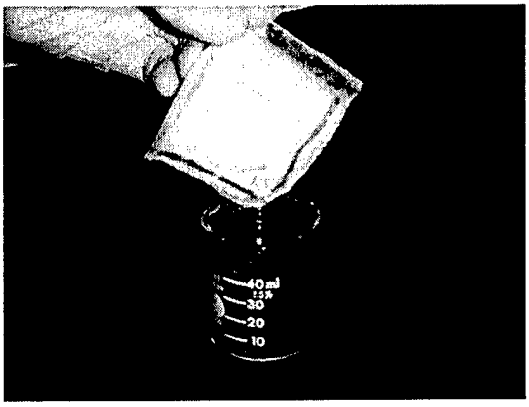


Figure 15.

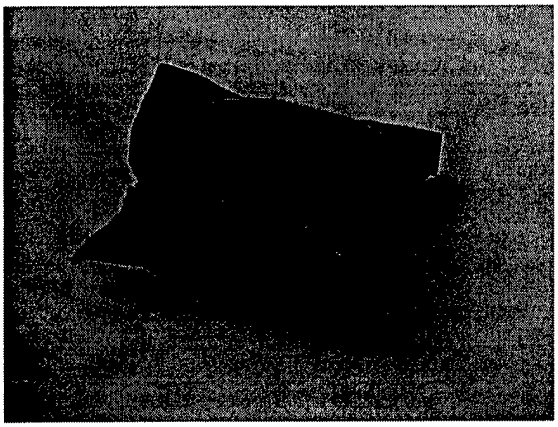
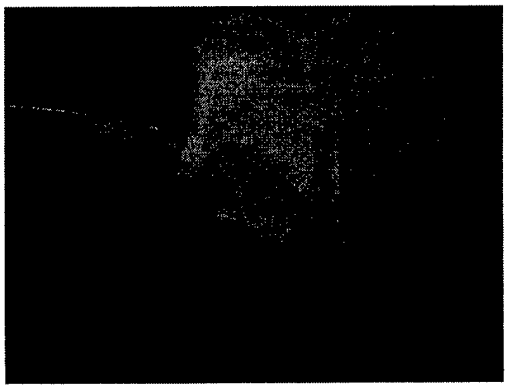


Figure 16.

Documentation of the ability of the device to develop force in a confined space (Goals 3 and 5)

Testing of prototype devices composed of outer EVA-EVOH composite shells containing Poly(acrylic-co-acrylamide) potassium salt were initially performed on a partially confined external limb model (figures 17-18). These measurements verified the ability to produce force (figure 19).



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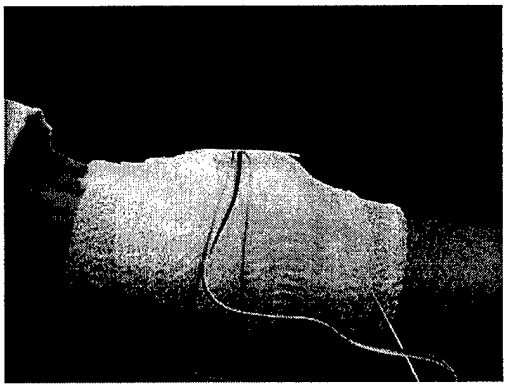

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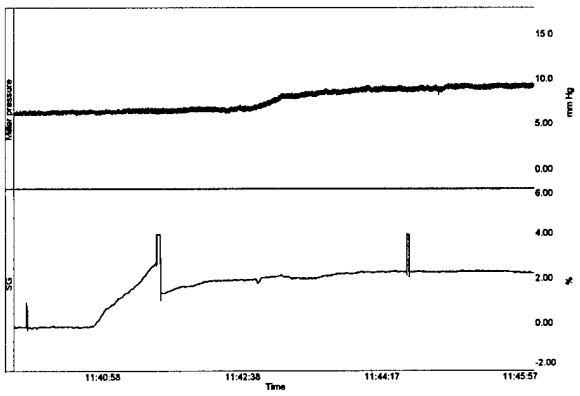

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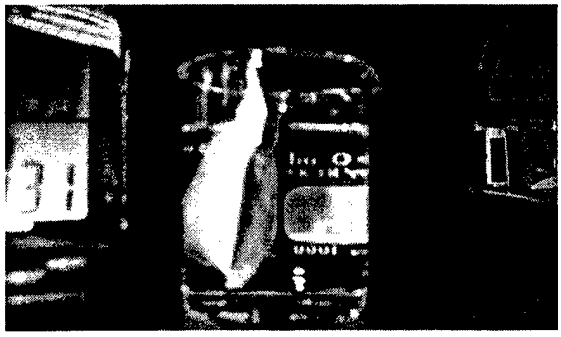

Figure 20. 31 seconds of swelling.

Further modification of the prototype allowed sealing of the device without the large flat edge caused by heat sealing. Testing of this model revealed rapid swelling within three minutes (figures 20-21) and rapid development of pressure in a true confined space model (figures 22-24). The goal of sufficient swelling to develop 90mm Hg pressure within 3 minutes was achieved.

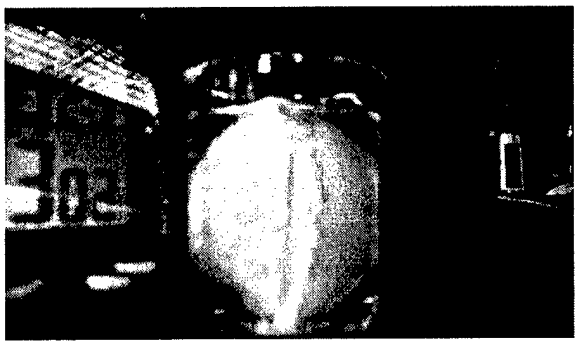

Figure 21. 182 seconds of swelling

Figure 22. Top View Before swelling

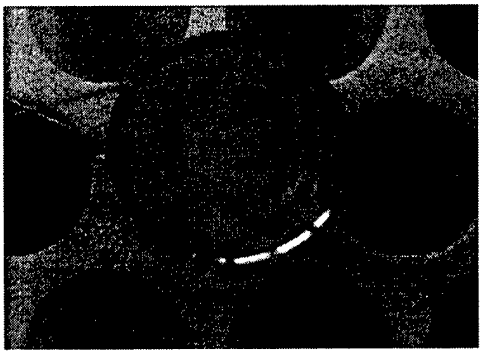

Figure 23. Top view after swelling.

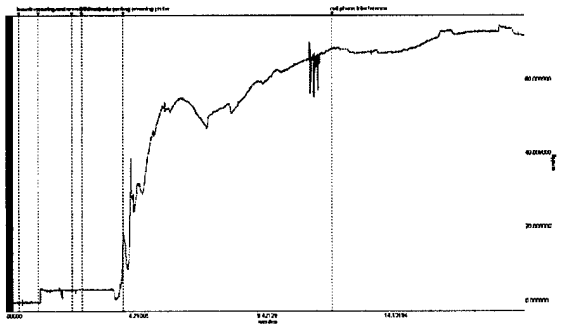

Figure 24. Pressure during swelling.

Swelling of device in whole blood continues to be more of a problem, and is being addressed in several ways. The first is to increase the pore size of the outer shell. A second is to alter the inner salt to improve its absorption of blood cellular elements. A third possible solution is to inject the device with sterile liquid and cause swelling without reliance on absorption of fluid moving in through the outer membrane. Since the injection rate determines the rate of pressure development, this type of device might allow even more rapid generation of pressure. The attachment of a pressure probe could also allow documentation of the pressure achieved and thus serve as a safe guard for overshoot. Finally, if a hemostatic agent is to be incorporated into the device the infusional liquid offers significant advantages. First the agent can be contained within the liquid and injected with the fluid. Second, since the fluid flow in the pneumatic device is from inside out, the fluid can be used to carry the hemostatic agent out into the space around the device. Third, if the hemostatic agent is on the outside of the device, one surface can be made of non-expansile material and the pressure within the device can be used to press the non-expanding, hemostatic agent coated surface up against the bleeding source. The device becomes a hand inside the wound pushing the hemostatic bandage against the area of bleeding. Given the multiple potential advantages of this embodiment of the biohemostat, fabrication and feasibility testing were performed (figures 25-26). The device rapidly swells, develops pressure that can be easily measured with a simple pressure guage, and remains "inflated" after removal of the syringe.

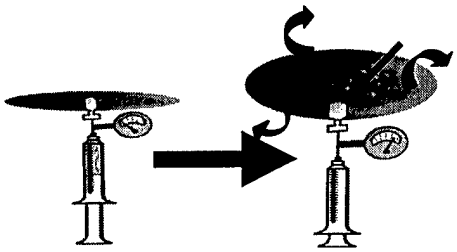

Figure 25. Schematic of pneumatic device.

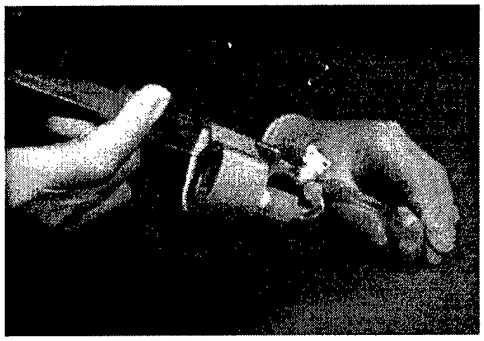

Figure 26. Injection of the prototype.

Addition of a hemostatic agent to the device to speed clot formation (Goals 4 and 8)

Thrombin was the first agent tested in the device. Initial attempts incorporated thrombin in the EVA-EVOH mat during electrospinning. At relatively low concentrations (5.6 NIH units of thrombin were electrospun into a 200 cm^2 EVA-EVOH mat), minimal shortening (120 to 110 seconds) of the clotting time was noted. When higher thrombin concentrations (50 to 100 units) were used the material spun with thrombin reproducibly shortened the whole blood clotting time.

Recently, several of the investigators on the Biohemostat project (Bowlin, Wnek, Carr, et al) were able to electrospin fibrinogen. The resulting polymer looks and behaves like fibrin (figure 27). In its dry state it is a fabric with the feel of silk gauze (figure 28). When exposed to liquid the material immediately (1-3 seconds) absorbs fluid and takes the characteristics of a fibrin clot. In preliminary animal experiments, the material produces hemostasis in seconds in a rat model of vascular bleeding.[42] Patents have been filed and the intellectual property license has been issued to

Nanomatrix Inc. Discussions between Nanomatrix Inc. and Hemodyne Inc. have produced a transfer of material agreement which will allow testing electrospun fibrinogen as the hemostatic agent on the Biohemostat. This will hopefully be accomplished at USAISR as outlined below. If successful, the two companies will proceed with a co-development agreement to produce the device.

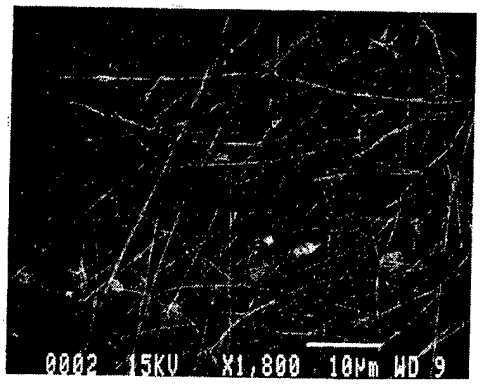

Figure 27. Electrospun fibrinogen (ESF).

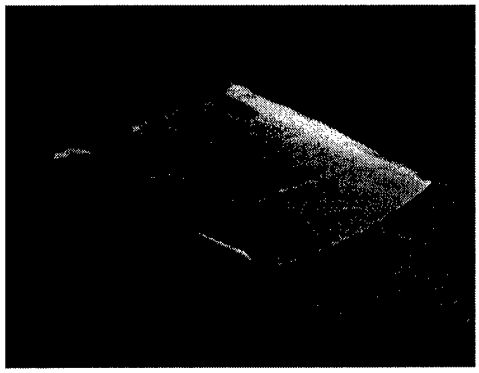

Figure 28. ESF guaze.

Initiate testing of the prototype device testing in a ballistic animal model (Goals 10 and 11)

Although our animal model is approved by the university IACUC and safety committees, we have been unable to initiate these studies for several reasons. Concerns have been raised about physical security at the VCU Sanger Hall Facility given the frequent appearances of animal rights activists, demonstrators and protestors at that location. Therefore, even though approved, the use of a large animal, ballistic injury model proved problematic at the MCV location. We next explored the possibility of moving this portion of the study to the Richmond VA Medical Center Animal studies facility. Conversations were initiated with the McGuire Research Institute of the Richmond VA who were willing to act as a "vendor" for the animal study portion of our project. Unfortunately, problems arose over potential intellectual property claims by the VA health care system. These were confirmed when the VA Central office issued a memorandum explictedly stating that any and all use of animal facilities at VA medical centers for research had to come under the standard VA Intellectual Property policy. A second problem has been the unanticipated expense of the bullet trap ($38,000) and the necessary modifications of the facility to accommodate this equipment. Potential solutions to these problems were discussed during a trip to the US Army Institute of Research (USAISR) in November of 2002. Several alternative animal models to test hemorrhage control devices are in place at USAISR, and they have the expertise to test our device in these models. Dr. Pusateri and Dr. Holcomb were very helpful and it now appears animal studies will begin later this year. Three short term goals are to: 1) establish and sign a CRADA, collaborative research and development agreement between USAISR and Hemodyne, Inc. the company who has licensed the intellectual property from VCU; 2) select the most appropriate animal model and adapt it to the needs of this study; and 3) arrange for the transfer of animal funds from the grant account at VCU to USAISR. To allow time to accomplish these delayed studies, a no cost extension of the current grant was sought and granted. Confirmation of the extension was received in late April of 2003. The extension is until April of 2004. Dr. Carr will be spending the

last two weeks of June of 2003 at USAISR to complete these arrangements listed above.

F. KEY RESEARCH ACCOMPLISHMENTS

- Demonstrated the utility of electrospinning in the "tailoring" of candidate polymers
- Tested the structural, chemical and performance characteristics of a variety of candidate polymers for the device outer shell
- Selected the outer polymer for the device
- Optimized the properties of the outer shell via a composite of two polymers
- Tested a variety of potential hydrophilic, super absorbent polymers
- Defined critical parameters for rapid swelling
- Selected the inner hydrophilic, hyper-absorbent polymer
- Optimized the properties of the inner polymer in terms of salt content and neutralization
- Constructed a variety of prototype devices
- Demonstrated the rapid swelling in water
- Defined the effects of increasing ionic strength, pH and plasma proteins on device performance
- Met engineering goal of rapid (within 180 seconds) production of significant (90 mm Hg) pressure in a confined space
- Demonstrated the feasibility of adding a hemostatic agent to the device
    - Incorporated thrombin into the device and demonstrated significant clotting activity
    - Developed a completely new electrospun hemostatic agent and arranged to test it in the Biohemostat device
- Proposed additional versions of the device which envision and will allow:
    - Rapid (within a few seconds) pneumatic force development (figures 25-26)
    - Incorporation of analgesics in the device
    - Incorporation of antibiotics in the device
    - Incorporation of other hemostatic agents in the device
    - Application to external wounds (figure 27)
- Constructed "pneumatic" Biohemostat device and demonstrated rapid force development

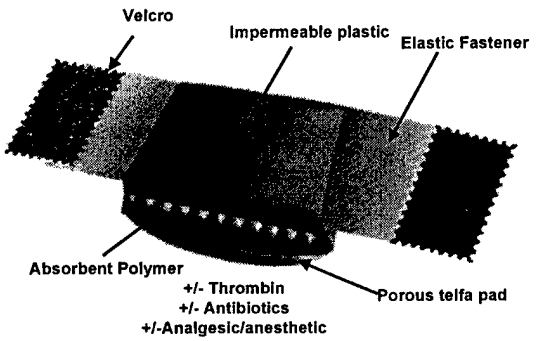

Figure 27. Schematic for external biohemostat.

G. REPORTABLE OUTCOMES

Manuscripts (three):

Layman JM, Kenawy E, Watkins JR, Carr ME, Bowlin G, Wnek GE. Development of the BioHemostat: a treatment modality for high pressure bleeding based on super-absorbent polymers and electrospun membranes. Submitted for publication.

Kenawy ER, Carr ME, Wnek G, Bowlin G. Performance characteristics of the BioHemostat – An engineering prespective of a clinical device being developed to treat high pressure bleeding. Manuscript in Preparation.

Ward K, Barbee W, Wnek G, Kenawy ER, Carr ME. Is pressure within the wound better than pressure around the limb for treatment of high pressure bleeding in trauma – can the tourniquet be replaced? Manuscript in preparation.

Abstracts (four):

Carr M, Wnek G, Cohen K, Ward K, Ivatory R, Bowlin G. The BioHemostat – Acute treatment modality for High Pressure Bleeding – Changing the Treatment Paradigm to: "Saving the Patient and Sparing the Limb." Proceedings of ATACCC 2001 – Advanced Technology Applications for Combat Casualty Care. Page 30, Abstract 44.

Carr M, Kenawy E, Layman J, Wnek G, Ward K, Barbee W, Tiba M. "Development of the BioHemostat, a treatment modality for high pressure bleeding based on super absorbent polymers." Presented at the twenty-fifth annual Conference on Shock of the Shock Society. Big Sky, Montana. June 8-11, 2002.

Carr M, Kenawy E, Layman J, Wnek G, Ward K, Barbee W, Tiba M. Development of the BioHemostat: a treatment modality for high pressure bleeding based on super absorbent polymers and electrospun bag." Proceedings of ATACCC 2002 – Advanced Technology Applications for Combat Casualty Care. Page 64, Abstract HC 18.

Layman JM, Kenawy E, Watkins JR, Carr ME, Bowlin G, Wnek GE. Development of the BioHemostat: a treatment modality for high pressure bleeding based on super-absorbent polymers and electrospun membranes. Submitted for presentation at the 2003 annual meeting of the American Chemical Society in New York, NY.

Presentations (four):

Carr, ME. "The BioHemostat – Acute Treatment Modality for High Pressure Bleeding" Presented at the Proceedings of ATACCC 2001 – Advanced Technology Applications for Combat Casualty Care. Ft. Walton Beach, FL. September 11, 2001.

Carr, ME. "Development of the BioHemostat Device for Control of High Pressure Bleeding" Presented to the Blood Research Group of the Walter Reed Army Institute of Research in Washington , DC. March 2002.

Carr, ME. "Development of the BioHemostat – A New Treatment for High Pressure Bleeding" Presented at the BIO-Defense & Homeland Security Procurement Conference in Washington, DC. April 30, 2002.

Carr, ME. "Development of the BioHemostat: a treatment modality for high pressure bleeding based on super absorbent polymers and electrospun bag." Presented at ATACCC 2002 – Advanced Technology Applications for Combat Casualty Care. St Pete Beach, FL. Sept 11, 2002.

Patent Applications (one):

"BioHemostat – a device for acute treatment of high pressure bleeding." Complete US and International Patent applications filed December 2002.

Invention disclosures (two):

"Bio-Hemostat – External Acute Wound Dressing" Filed 31 August 2001.

"Pneumatic Bio-Hemostat – Rapid Pressure Development for Treatment of High Pressure Bleeding" Filed 31 August 2001.

Funding applied for (one):

Pre-Proposal submitted to the Army for development of the electrospun fibrinogen bandage.

Application to NIH for development and testing of electrospun fibrin bandage.

H. **CONCLUSIONS** – Work accomplished to date indicates that the underlying premise of this project is sound. A prototype device has been fabricated which rapidly swells and produces force. The ability to accomplish similar results in blood has been demonstrated at least in principle. Since we are not committed to any particular hemostatic agent, the potential to incorporate (or at least test) a variety of candidate materials is obvious. To this point, the expense of the device remains minimal, and it should be relatively simple to mass-produce a similar product. The potential for utilizing the hydrophilic polymer to not only apply direct pressure but to also serve as a drug delivery system for analgesics and antibiotics (both internally and externally) holds promise not anticipated in the original application (figure 20). The uniqueness of this approach is obvious and to this point the results have been gratifying.

Animal testing which will begin in the near future will be critical to the ultimate goal of moving this potential treatment into human trials. If development continues along its current trajectory one would anticipate that this device should have utility in both compressible and non-compressible bleeding. If this is correct, it will be superior to all currently available treatments, and tourniquets will no longer be required. The treatment paradigm will have shifted from save the patient and then worry about the limb to save the patient and preserve the limb. In the process, the patient and the initial care provider will have a better treatment for the primary cause of death in trauma.

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Development of the BioHemostat – a treatment modality for high pressure bleeding based on super-absorbent polymers and electrospun membranes

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Introduction

Ballistic injury is a primary mode of trauma in combat. Such injuries can be associated with rapid blood loss due to vascular disruption. In the Vietnam conflict, ten percent of wounds to the extremity were associated with major artery injuries.[1] While bleeding from compressible vessels may respond to direct pressure, blood loss from deep muscular branches, such as those from the profunda femoris artery, may be severe.[2] Despite increasingly aggressive surgical treatment, limb salvage has not improved,[3] and death from hemorrhagic shock remains a problem even in very healthy individuals.[4] Combat vascular injuries continue to result in a 12 to 30 percent amputation rate depending on the involved vessel.[5]

Recent developments in the field of hemostatic agents have raised the possibility of alternative treatment for vascular bleeding. The development of virally inactivated fibrin sealant and its documentation as a useful adjuvant to multiple types of surgery have been major advances.[6,7] However, there remains a need for a tourniquet alternative that is inexpensive, lacks viral risk, and can be easily administered by an army medic or civilian EMT. We introduce here an approach under development in our laboratories that in principle meets these criteria.

The BioHemostat Concept.

The BioHemostat is a conceptually simple device that, when exposed to aqueous solutions, rapidly develops pressure in a confined space. Our first-generation device is composed of a flexible, porous outer membrane that confines hydrophilic, super-absorbent polymer. The latter is a cross-linked, hydrophilic polymer that has the ability to absorb large quantities of pure water, saline or physiological solutions. The super-absorbent polymers used in our studies are cross-linked poly(acrylic acid) salts and copolymers. The highly porous 'bag' or shell is made from electrospun polymers and possesses the dual functionality of having high quality water permeability and elasticity to accommodate the swelling of the polymer hydrogel that resides inside. Additionally, the bag must contain the gel with minimal leakage. The ultimate goal is to optimize this device for placement directly into a wound to develop counter pressure to aid in hemorrhage control, while avoiding the crush injuries and ischemic damage associated with tourniquet use.

The electrospinning process is an attractive approach for fabricating the shell of our device, as it offers not only the opportunity for control over material morphology, porosity, and composition, but also utilizes a simple experimental set-up. In electrospinning, polymer solutions or melts are deposited as fibrous mats onto a predetermined substrate. Fibers are produced by charging the liquid polymer solution to 5-30 kV versus a grounded target a short distance away. The jet resists break-up into droplets due to polymer chain entanglements afforded by high concentration polymer solutions. Such fibrous materials may be useful for many applications in medicine, such as wound dressings and scaffolds for tissue engineering.[8] Due to their biocompatibility, ethylene-vinyl acetate (EVA) and ethylene-vinyl alcohol (EVOH) copolymers were selected as bag materials. Both have been recently electrospun in our laboratories[9,10] and we have demonstrated controlled release of antibiotics from electrospun EVA.[9] We find that EVA and EVOH have complementary mechanical and wetting properties and frequently employ both characteristics in BioHemostat bags to take simultaneous advantage of these properties.

The swelling or prototype devices in water, normal saline, and human blood and dehydration kinetics of the selected super-absorbent polymers and the prototype device have been investigated, although only preliminary swelling results are discussed here.

Experimental

Materials. Poly(ethylene-co-vinyl acetate), EVA (Elvax 40W, 40 wt% vinyl acetate, M_w = ca. 60 kD, T_g ca. -36°C), was a gift from DuPont. EVA pellets were soaked in ethanol for several days to remove antioxidants. Poly(ethylene-co-vinyl alcohol), EVOH, was a gift from Soarus, with compositions of 56-71 mol % vinyl alcohol. 2-propanol (isopropanol) was obtained from LabChem Inc., and 70/30% v/v isopropanol/water for EVOH electrospinning was prepared by addition of deionized water. Chloroform was purchased from Aldrich and used as received. Crosslinked poly(acrylic acid) potassium salt was supplied by BASF. Poly(acrylic acid-co-acrylamide) potassium salt (crosslinked) was purchased from Aldrich.

Preparation of electrospun bags. Electrospinning of EVA. Electrospinning was carried out using 13 % w/w solutions of EVA in chloroform. The electrospinning set-up, shown in **Figure 1**, consisted of a syringe and needle as one electrode, a counter grounded electrode which was a stainless steel rotating drum with variable speed control, and a high voltage power supply (model CZE1000R, Spellman). The charged needle was located approximately 20 cm from the grounded target. A positive voltage of 15 kV was applied to the polymer solution using an alligator clip attached to the stainless steel needle. The solution was delivered via a syringe pump to

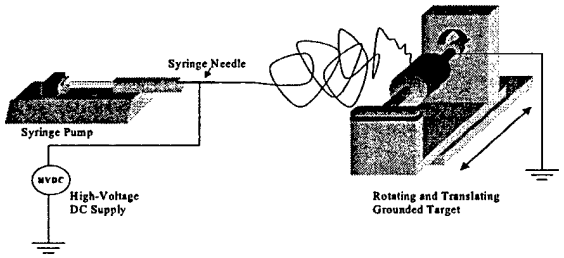

Figure 1. Schematic of the electrospinning system

control a volumetric flow rate of 12 ml/h. The resulting fibers were collected on the rotating metal drum with a speed of ca. 1000 rpm to produce a sheet of non-woven fabric. The size of the produced mat is typically 10 x 20cm.

Electrospinning of EVOH. The procedure for EVA was similar to that described for EVA except for the solvent was isopropanol/water (70/30 v/v). Solutions of EVOH and 2-propanol/water at 10w/v % were prepared by heating the polymer mixture to about 75°C until a homogeneous solution was obtained. The EVOH solutions were cooled to room temperature and then electrospun utilizing the aforementioned processing conditions.

Fabrication of a BioHemostat device. 'Tri-layer' mats were prepared by first electrospinning EVOH followed by an overcoat of EVA fibers and finally by an additional layer of EVOH fibers. The total thickness of the tri-layer mats ranged from 0.3-0.4 mm. The tri-layer mats were first sealed on 3 sides using a heat sealer, although cyanoacrylate adhesive could also be used to seal the edges. The super-absorbent polymer, cross-linked poly(acrylic acid) salts or copolymers, was added to the bag prior to closure of the end seam. Typical device dimensions were 5 x 3 cm.

Swelling of BioHemostat devices. Hydration studies were performed to determine the degree of swelling. A known weight of the dry, super-absorbent copolymer was incorporated into the bag made of the electrospun EVA and EVOH. Devices were immersed into a beaker containing 100 ml of deionized water. The approach to equilibrium was monitored by periodically withdrawing the bag from the water, removing the excess surface water by light blotting with filter paper, and then determining the weight. Each device weight was monitored until a constant mass was observed. The absorbency of each device was calculated using following equation:

$$\text{Absorbency (g/g)} = W_2\text{-}W_1/W_1$$

where absorbency is expressed in grams of water retained per gram for the dried gel, and W_1 and W_2 are the weight of the dry and swollen super-

absorbent polymer, respectively. Photographs of a typical as-prepared and a water-swollen device are shown in **Figure 2.**

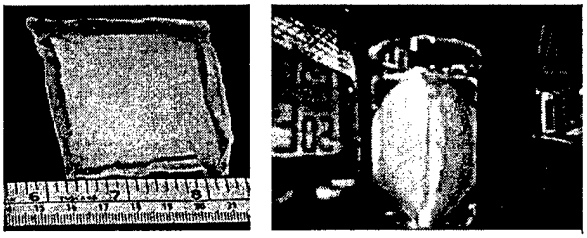

Figure 2. Left: 'tri-layer' bag with small amount of dry, crosslinked poly(acrylic acid) K+ salt. Right: same device after 3 minutes in water.

Dehydration of BioHemostat devices. Dehydration studies were executed after the hydrated device reached a constant weight, as described in the previous section. For these studies, the device was removed from the water and the weight loss recorded until the original dry weight of the bag or a new constant weight was obtained.

Results and Discussion

In our first-generation devices, the outer membrane consisted of only electrospun EVA. The elasticity of EVA is desirable as the device will swell to several times its original size. However, the hydrophobicity of EVA yielded unsatisfactory swelling rates; bags made from electrospun EVA took several tens of minutes to reach maximum swelling. To mitigate this problem, electrospun EVOH was exploited for its hydrophilicity. Although EVOH satisfied the wettability requirement, the elasticity of the polymer is relatively poor for this application. Hence, the construction of a hybrid, 'tri-layer' membrane proved to capture both requirements of water affinity and elasticity. Typical deionized water absorbency curves for such a tri-layer bag with either the potassium salt of poly(acrylic acid-co-acrylamide) or poly(acrylic acid) inside are shown in **Figure 3.**

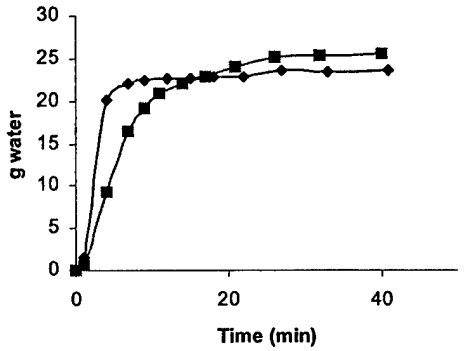

Figure 3. Typical water absorbency curves of 'tri-layer' bags with crosslinked poly(acrylic acid-co-acrylamide) or poly(acrylic acid) K^+ salt as the super-absorbent polymer; the curve showing the more rapid uptake of water is for a bag containing poly(acrylic acid).

Dehydration studies aimed to study the water retention by the BioHemostat. The device took, on average, 15 days for it to be completely free of water. Water retention remained high for the first 5 days, which would be appreciated in circumstances where immediate health care was unavailable.

Conclusions

The VCU BioHemostat may present a viable alternative to hemorrhage treatment. The biocompatibility, simplicity, and quick response of the device make it an attractive replacement to the routinely used tourniquet. We are currently quantifying swelling kinetics and pressure development using whole blood, and are optimizing swelling kinetics and mechanical properties.

Acknowledgments. We thank the U. S. Army Medical Research and Procurement Office for support of this research and BASF, Soarus and Dupont for generous supplies of materials.

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Development of the BioHemostat
A Treatment Modality for High Pressure Bleeding
Based on Super Absorbent Polymer and Electrospun Bag

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ABSTRACT

Bleeding from an artery is difficult to control due to the high pressures found in the arterial system. Hemorrhage is especially problematic in penetrating wounds where the bleeding source may not be apparent. Tourniquets that are routinely used to treat such wounds can cause multiple complications. We are developing a device which, when exposed to aqueous solutions, rapidly generates pressure in a confined space. In this report, we summarize the design and testing of a prototype device. The "biohemostat" is composed of a flexible outer membrane which surrounds a hydrophilic, super-absorbent polymer. The outer bag is made from an electrospun mat of Ethylene-vinyl acetate co-polymer. The electrospun mat is very flexible, durable (stretching to 10 times its original length prior to tearing), biocompatible and porous. Its relative degree of hydrophobisity is overcome by incorporating a percentage of EVOH either as a blend or composite. The hydrophilic polymer used in the prototype device is composed of polyacrylic acid derivatives or copolymers. When the device is placed in aqueous solutions it rapidly absorbs fluid, expands and develops significant pressure in a confined space. Although swelling of such polymers is dependent on the nature of the aqueous solution (i.e.. Varies with pH, ionic strength, protein content, etc.) the decreases in absorption caused by these parameters has been easily overcome by increasing the amount of hydrophilic polymer. The goal is to develop a device, which can be placed directly into a wound to develop counter pressure to aid in hemorrhage control. By developing pressure directly on the bleeding site, the hope is to avoid the crush injuries and ischemic damage associated with tourniquet use.

INTRODUCTION

Ballistic injury is a primary mode of trauma in combat. Such injuries can be associated with rapid blood loss due to vascular disruption. In the Vietnam conflict, ten percent of wounds to the extremity were associated with major artery injury.[1] While bleeding from compressible vessels may respond to direct pressure, blood loss from deep muscular branches such as those from the profunda femoris artery may be severe.[2] Despite increasingly aggressive surgical treatment, limb salvage has not improved[3], and death from hemorrhagic shock remains a problem even in very healthy individuals.[4] Combat vascular injuries continue to result in a 12 to 30 percent amputation rate depending on the involved vessel.[5]

Described more than 2000 years ago as an adjuvant to surgical amputation[6], tourniquets have become a primary initial treatment of injuries with associated high pressure bleeding. Unfortunately, tourniquet utilization can be associated with a variety of complications including nerve injuries, distal ischemia, compartment syndromes, post-tourniquet syndrome, and pulmonary embolus.[7,8] A major consequence of these complications is an increased risk of limb wastage. Despite these potential complications, combat as recent as the 1991-92 Croatian conflict has verified the ability of tourniquets to delay shock in lower extremity arterial injuries.[9] Recent developments in the field of hemostatic agents have raised the possibility of alternative treatment of vascular bleeding.

The development of virally inactivated fibrin sealant and its documentation as a useful adjuvant to multiple types of surgery have been major advances.[10,11] The effectiveness of fibrin glue in speeding hemostasis along vascular graft suture lines[12], presaged its testing as an adjuvant to surgery in the treatment of complex hepatic injury.[13] Alternate formulations of fibrinogen and thrombin containing dressings[14] and dry fibrin sealant dressings[15] have prompted studies of these dressings in pig models of vascular injury[14] and grade V liver injury.[15,16] Dry fibrin sealant dressing was recently shown to be more effective than standard gauze in decreasing bleeding and maintaining blood pressure in ballistic injury.[17]

While the development of "dry" products has increased their potential as alternatives to tourniquets for battlefield treatment, several potential problems remain. First, these products are very expensive. Second, although virally inactivated, the fibrinogen they contain comes from multiple human donors and cannot be considered totally safe in terms of pathogen transmission. Third, these products must be held in place until bleeding stops or the material may simply wash out of the wound. This is especially true when the bleeding is brisk as with arterial involvement. The need for a tourniquet alternative that is effective, inexpensive, lacks viral risk, and can be easily administered by an army medic is obvious.

Superabsorbent polymers are crosslinked hydrophilic polymer networks with the ability to absorb large quantities of pure water, saline or physiological solutions.[18,19]

Superabsorbent polymers can absorb large amounts of water or other fluids and swell up to thousands of times their own weight in aqueous media.
The absorbed water is retained within the network even under considerable pressure.[20,21] Superabsorbent polymers have been utilized in a variety of applications including drug delivery systems, absorption pads, consumer care products, disposable diapers, hygienic napkins, biomedical materials, soft contact lenses, supports for catalysts, soil components for agriculture and horticulture, gel actuators, water blocking tapes, and artificial snow for winter sports.[22-25]
The electrostatic spinning (electrospinning) process is an attractive approach for processing polymer biomaterials because it offers the opportunity for control over material morphology, porosity, and composition using simple equipment. In electrospinning, polymer solutions or melts are deposited as fibrous mats rather than droplets. At sufficiently high polymer concentrations, chain entanglementsin melts allow production of continuous fibers. The fibers are produced by charging the liquid to 5000-30,000 Volts vs. a ground a short distance away. This leads to injection of the charged liquid from the catheter type electrode and capture of the forming polymer on a device placed between the catheter and the electrical ground.

Electrospinning is a cost effective method for producing fibrous polymer mats with fiber diameters ranging from 0.01 μm to several tens of μm.[26-29] Such materials may be useful for many applications in medicine such as wound dressings and scaffolds for tissue engineering.[30-32] The simplicity of the electrospinning process itself, the ability to control the fiber diameter and overall porosity of the resulting mat, and the ability to incorporate therapeutic compounds into the mats during spinning afforded the prospect of preparing useful polymer systems for controlled drug delivery. While flat mats represent an attractive form for topical delivery applications, other shapes (e.g., tubes) can be can be constructed using different target geometries.

We used the electrospinning technique to manufacture the outer (highly permeable) bag of the biohemostat device. We had previous shown the utility of this approach in the delivery of drugs such as the antibiotic tetracycline Hydrochloride.[33]
Due to its well know biocompatibility, ethylene-vinyl acetate (EVA) coplymer was selected for these biohemostat applications.[34-36] EVA has been used in many biomedical applications including controlled release of drugs and macromolecules such as immunoglobulin G.[37,38] In some applications, such as controlled release of insulin, EVA has been used as an implantable polymer.[39] Its use in the field of dental diseases has also been reported.[40,41]
In this report, we detail work accomplished during the first year of a grant (DAMD 17-01-1-0691) from the United States Army. Progress toward the design and construction of a new hemostatic device composed of a porous outer bag containing superabsorbent polymers is summarized.

METHODS AND RESULTS

The original device concept involved a relatively small, flexible bandage that would be placed directly in the wound. Once in place, liquid within the wound would rapidly penetrate the bandage and be absorbed by super-absorbent polymers contained within the bandage. As a consequence of fluid absorption, the bandage would expand and develop pressure within the wound to aid in hemorrhage control.

Development of the Outer (encasement membrane) Material
Based on this simple design, the outer material of the device had to meet certain functional criteria. The outer coating must be: flexible, porous, biocompatible, wettable, and durable. We chose electrospinning (Figure 1) as the process by which we would prepare various sheets of candidate polymers for initial testing. Electrospinning is simple, inexpensive and allows the production of polymer sheets of varying size, thickness and shape. Ethylene Vinyl Acetate (EVA) was initially chosen as a candidate material due to its high flexibility (figure 2), large pore size (see figure 3) and biocompatibility. When viewed by scanning electron microscopy, electrospun EVA is composed of a continuous string of polymer (i.e. there are no obvious polymer endpoints). This property increases structural integrity and therefore decreases the possibility of device rupture during use or removal.

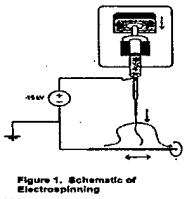


Figure 1. Schematic of Electrospinning
Figure 1

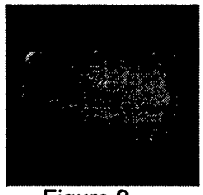


Figure 2

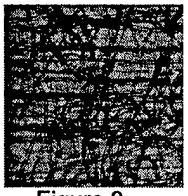


Figure 3

Unfortunately, EVA is not extremely wettable. This property slowed initial swelling of prototype devices. Once swelling began, it proceeded rapidly due to the opening of large pores in the EVA network. The period prior to rapid swelling was too excessive to allow EVA to function as the outer device material.
Addition of hydroxyl (OH) groups to EVA produces Ethylene Vinyl Alcohol (EVOH), which is dramatically more wettable but much less flexible. By combining alternatinglayers of EVA and EVOH a material was produced with the requisite outer-shell properties (see figure 4).

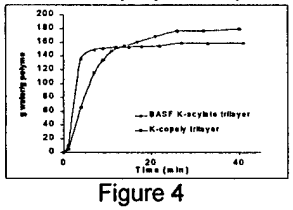


Figure 4

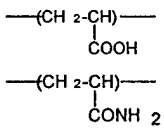


Figure 5

Selection and optimization of Super-Absorbent Inner Polymer Materials
Testing of several candidate commercially available super-absorbent polymers identified poly(acrylic-co-acrylamide) (Figure 5) as an appropriate hydrophilic polymer for the BioHemostat device. The speed of swelling was optimized by the addition of potassium salt (Figure 6) and by polymer neutralization (figure 7).

Direct comparison of the neutralized polymer and the potassium salt (Figure 8) lead to the acceptance of the Poly(acrylic-co-acrylamide) potassium salt as the best candidate for further device development. Thispolymer rapidly expands, absorbing up to 1,000 times its weight in water (figures 10 and 11).

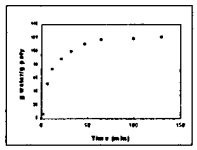


Figure 6

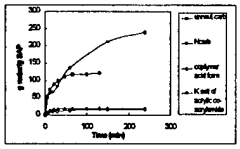


Figure 7

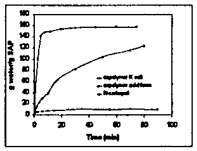


Figure 8

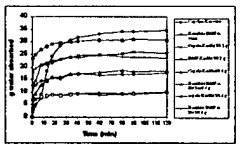


Figure 9

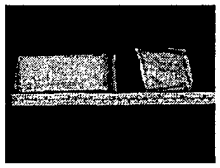


Figure 10

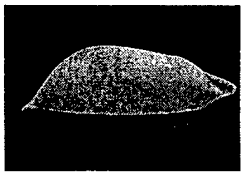


Figure 11

The absorption characteristics of all super-absorbent polymers are to some degree dependent on the nature of the aqueous environment. That is to say, absorption is altered by increasing ionic strength, changes in pH and the presence of proteins. Since the BioHemostat will be utilized at an ionic strength of 0.15M, a pH of 7.4 and in the presence of large amounts of protein, the ability of prototype devices to absorb salt solutions, plasma and blood were therefore studied. As anticipated, increased ionic strength and the presence of protein slowed and decreased absorption. This was most easily overcome by simply increasing the amount of absorbent polymer contained within the devices (figure 9). Subsequent testing in whole blood (figures 12 and 13) confirmed the ability of the device to absorb in this environment.

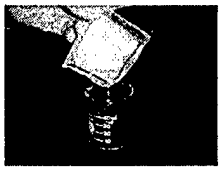


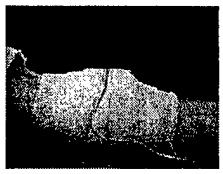
Figure 12

Figure 13

Documentation of the ability of the device to develop force In a confined space

Testing of prototype devices composed of outer EVA-EVOH
composite shells containing Poly(acrylic-co-acrylamide) potassium salt has recent verified their capacity for force generation in a simple confined space model (figure 14). Additional studies are ongoing to optimize this ability as animal trials are approached.

Figure 14.



Addition of a hemostatic agent to the device to speed clot formation

Thrombin is the only agent tested to this point. Attempts have been made to incorporate thrombin in the EVA-EVOH mat during electrospinning. These have been minimally successful to this point. A minimal shortening (120 to 110 seconds) of the clotting time was noted when 5.6 NIH units of thrombin were electrospun into a 200 cm^2 EVA-EVOH mat. We are convinced that this simply represents the use of too little thrombin. Ongoing experiments will incorporate several hundred units of thrombin on a similar piece of material.

CONCLUSIONS

Work accomplished to date indicates that the underlying premise of this project is sound. A prototype device has been fabricated which rapidly swells and produces force. The ability to accomplish similar results in blood has been demonstrated at least in principle. Since we are not committed to any particular hemostatic agent, the potential to incorporate (or at least test) a variety of candidate materials is obvious. To this point, the expense of the device remains minimal, and it should be relatively simple to mass-produce a similar product. The potential for utilizing the hydrophilic polymer to not only apply direct pressure but to also serve as a drug delivery system for analgesics and antibiotics (both internally and externally) holds promise not anticipated. If development continues along its current trajectory one would anticipate that this device should have utility in both compressible and non-compressible bleeding. If this is correct, it will be superior to all currently available treatments, and tourniquets will no longer be required. The treatment paradigm will have shifted from save the patient and then worry about the limb to save the patient and preserve the limb. In the process, the patient and the initial care provider will have a better treatment for the primary cause of death in trauma.

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